Recombinant DNA Technique and Sickle Cell Anemia Research

Letters from:

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The data presented in the report by Allyson Cole-Strauss *et al.* (6 Sept., p. 1386) infer a remarkable phenomenon: 50 to 80% of mutant, β^S-globin loci in a population of B cells were converted to wild-type alleles after exposure of those cells to oligonucleotides containing wild-type, β^A, sequences. This represents an absolute recombination frequency (recombinant cells/exposed cells) that is three to six orders of magnitude higher than that normally seen in cultured mammalian cells (1).

These data were published without the clonal isolation of a single recombinant cell line. Gene conversion was assayed on pooled-cell extracts containing a mixture of reagent oligonucleotides and chromosomal DNA in which the wild-type/mutant sequence ratio approached $10^8/1$. Under such conditions, the potential for assay artifact should be considered, yet neither a zero time point nor an end point, in the form of cloned cells, was performed.

The implications of this data should demand the utmost in experimental control.

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References

1. Thomas, K., Folger, K., Capecchi, M., *Cell* **44**, 419 (1986) ; Thomas, K., Capecchi, M., *ibid.* **51**, 503 (1987) .

Response: We appreciate the timely comments of Thomas and Capecchi and thank them for their important suggestions. The observations that we described in our report (1) are the initial findings of an ongoing study in which established, stable cell lines, altered genetically by chimeric oligonucleotides, are now being grown out. For all efforts, numerous controls are performed to eliminate alternative explanations, such as polymerase chain reaction artifacts, as the basis for the observations. A number of avenues of investigation opened by our observations are currently being pursued.

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References

1. Cole-Strauss, A. *et al.*, *Science* **273**, 1386 (1996) .

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